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McCord et al.

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(54) **MATERIALS AND METHODS FOR ACHIEVING DIFFERENTIAL LYSIS OF MIXTURES WITH THE AID OF ALKALINE LYSIS AND PRESSURE CYCLING TECHNOLOGY (PCT)**

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CPC ..... **C12N 1/06** (2013.01); **C12N 15/1003** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

#### **U.S. PATENT DOCUMENTS**

2009/0286301 A1\* 11/2009 Tao ..... C12N 1/066  
435/198

#### **OTHER PUBLICATIONS**

Wilson, Kate, "Preparation of Genomic DNA from Bacteria," Current Protocols in Molecular Biology, 1997, pp. 2.4.1-2.4.5.\*

Hudlow et al., "Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence," *Forensic Science International: Genetics*, 2012, vol. 6, pp. 1-16.\*  
Baird, M. et al., "Allele Frequency Distribution of Two Highly Polymorphic DNA Sequences in Three Ethnic Groups and Its Application to the Determination of Paternity," *Am J Hum Genet*, 1986, 39:489-501.

Benschop, Corina C.G. et al., "Post-coital vaginal sampling with nylon flocked swabs improves DNA typing," *Forensic Science International: Genetics*, 2010, 4:115-121.

Burger, Joachim et al., "DNA preservation: a microsatellite-DNA study on ancient skeletal remains," *Electrophoresis*, 1999, 20:1722-1728.

Elliott, K. et al., "Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides," *Forensic Science International*, 2003, 137:28-36.

Fondevila, M. et al., "Challenging DNA: Assessment of a range of genotyping approaches for highly degraded forensic samples," *Forensic Science International: Genetics Supplemental Series*, 2008, 1:26-28.

Gill, Peter et al., "Forensic application of DNA 'fingerprints,'" *Nature*, Dec. 1985, 318(12):577-579.

Horsman, Katie M. et al., "Separation of Sperm and Epithelial Cells in a Microfabricated Device: Potential Application to Forensic Analysis of Sexual Assault Evidence," *Anal. Chem.*, 2005, 77:742-749.

Hudlow, William R. et al., "Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence," *Forensic Science International: Genetics*, 2012, 6:1-16.  
Jeffreys, Alec J. et al., "Hypervariable 'minisatellite' regions in human DNA," *Nature*, Mar. 7, 1985, 314:67-73.

Johanisson, E. et al., "Evaluation of 'round cells' in semen analysis: a comparative study," *Human Reproduction Update*, 2000, 6(4):404-412.

Kirkman-Brown, Jackson et al., "Evaluation of a disposable plastic Neubauer counting chamber for semen analysis," *Fertility and Sterility*, Feb. 2009, 91(2):627-631.

Norris, Jessica V. et al., "Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis," *J Forensic Sci*, Jul. 2007, 52(4):800-805.

Pang, B.C.M. et al., "Double swab technique for collecting touched evidence," *Legal Medicine*, 2007, 9:181-184.

Raymond, Jennifer J. et al., "Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes," *Forensic Science International: Genetics*, 2009, 4:26-33.

Smejkal, Gary B. et al., "Increased Protein Yields from *Escherichia coli* Using Pressure-Cycling Technology," *Journal of Biomolecular Techniques*, 2006, 17:173-175.

(Continued)

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(57) **ABSTRACT**

The subject invention provides a two-step protocol using pressure cycling technology (PCT) and alkaline lysis for differential extraction of mixtures. In a preferred embodiment the procedure is used in forensic DNA applications such as, for example, DNA testing in the case of rape.

**14 Claims, 5 Drawing Sheets**